Second-line treatment with dasatinib in patients resistant to imatinib can select novel inhibitor-specific BCR-ABL mutants in Ph+ ALL

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Resistance to targeted treatment with the BCR-ABL inhibitor, imatinib, in patients with chronic myeloid leukaemia and Philadelphia-positive (Ph+) acute lymphoblastic leukaemia can occur through the selection of point mutations within the ABL kinase domain, which stop imatinib binding without impairing kinase activity.1 Dasatinib (BMS-354825) is a second-generation tyrosine kinase inhibitor currently in clinical development for imatinib-resistant or imatinib-intolerant chronic myeloid leukaemia and Ph+ acute lymphoblastic leukaemia.2 In vitro assays3,4 and crystallographic studies5 have shown that the less stringent conformational requirements for BCR-ABL binding render dasatinib active against many of the ABL kinase domain mutations seen in patients who are resistant to imatinib. However, novel, inhibitor-specific mutants of BCR-ABL are thought to eventually emerge.6 Here we report two patients in whom resistance to dasatinib was associated with the emergence of two such dasatinib-specific mutants. Both patients were enrolled in a phase II programme of dasatinib for Ph+ leukaemia resistant to, or intolerant of, imatinib. The first case is an 18-year-old patient with Ph+ acute lymphoblastic leukaemia who had stopped imatinib because of disease recurrence. Mutation analysis at the time of relapse had indicated the presence of a Y253H BCR-ABL mutation, which resulted in resistance to imatinib. Dasatinib treatment was started in May, 2005, at a dose of 70 mg twice a day. The patient showed complete haematological and cytogenetic (0% Ph+ marrow metaphases) response after 3 months of treatment, and only wild-type Bcr-Abl was detectable by direct sequencing at 3, 6, and 9 months. At 12 months, mutation analysis indicated presence of BCR-ABL-positive cells harbouring an ACT to GCT nucleotide change (T315A), which coexisted with wild-type BCR-ABL-positive cells (superimposition of A and G peaks). At time of relapse (13 months), mutated cell population had outgrown to represent almost 100% of BCR-ABL-positive cells (G peak only). Dasatinib was stopped and rechallenging with imatinib at a dose of 800 mg per day was attempted. After 1 week from the start of imatinib, white blood-cell count had reduced from 45×10⁹/L to 1·9×10⁹/L. Mutation analysis done after 3 weeks and 6 weeks of imatinib showed that the T315A mutation was still present, and that additional G250E and F317L mutations had been selected. For this reason, and because of disease persistence, imatinib was withdrawn after 50 days.

The second case is a 74-year-old patient with Ph+ acute lymphoblastic leukaemia who had discontinued imatinib because of grade IV non-haematological toxic effects. Mutation analysis of a blood sample obtained before the onset of treatment with dasatinib had not shown evidence of ABL kinase domain mutations. Dasatinib was started in January, 2006, at a dose of 140 mg per day. The patient showed complete haematological response after 1 month and a complete cytogenetic response after 3 months of dasatinib treatment. At 6 months, the patient relapsed and sequence analysis detected a TTC...
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(Thymine, thymine, cytosine) to ATC (adenine, thymine, cytosine) nucleotide change, resulting in a phenylalanine to isoleucine amino acid substitution at residue 317 (F317I; figure 2). The patient died of acute pulmonary oedema 4 days after dasatinib discontinuation.

Threonine 315 and phenylalanine 317 are crucial contact residues both for imatinib and for dasatinib, but whereas the T315I and F317L amino acid substitutions are frequently reported in patients who are resistant to imatinib, neither the T315A or the F317I variants have ever been implicated, in vitro or in vivo, in imatinib resistance. However, the T315A and the F317I variants among others have been reported to have emerged in an in vitro saturation mutagenesis screening for BCR-ABL mutant forms that confer resistance to dasatinib.6 Ba/F3 cells expressing BCR-ABL with the T315A substitution were shown to have a 90 times higher half maximal inhibitory concentration (IC50) than those expressing wild-type BCR-ABL when incubated with dasatinib.6 Notably, the increase in imatinib IC50 for this mutant was only by 2-4 times, suggesting that in such a case resuming imatinib alone or in combination might prove effective. In our first patient, imatinib was resumed after dasatinib withdrawal, but the rapid selection of additional G250E and F317L mutations compromised its efficacy and made assessment of the actual in vivo activity of imatinib against T315A impossible. Similar in vitro data for the differential sensitivity of BCR-ABL with the F317I substitution to dasatinib and imatinib are currently unavailable, and unfortunately the patient died before imatinib could be readministered. An aurora kinase inhibitor (MK-0457, also known as VX-680) has been reported to be effective in patients with chronic myeloid leukaemia and Ph+ acute lymphoblastic leukaemia who harbour the T315I substitution.7 Cocrystal studies have shown that threonine 315 and phenylalanine 317 are not directly involved in MK-0457 binding,7 evidence which suggests that MK-0457 is probably active against both T315A-BCR-ABL and F317I-BCR-ABL.

Our two patients provide evidence that novel, inhibitor-specific mutations can actually be selected, although they do not seem to be common (in our experience, they were noted in two of 21 patients who were resistant to dasatinib). Moreover, these mutations raise concerns about the limitations of single-agent treatment in the long-term control of Ph+ acute lymphoblastic leukaemia, where the high rate of genomic instability can foster the development, over time, of multiple mutations within the same or in different BCR-ABL-positive cells, which will then be selected or de-selected depending on the spectrum of sensitivity and resistance to the inhibitor used.

Conflicts of interest
We declare no conflicts of interest.

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References


Figure 2: Results of sequence analysis of second patient at time of relapse, showing TTC to ATC nucleotide change (F317I).